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(71) Applicants: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 300 Lakeside Drive, 22nd Floor, Oakland, CA 94612-3550 (US). SCRIPPS CLINIC AND RESEARCH CENTER [US/US]; 10666 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors: SHEPPARD, Dean ; 1006 Hubert Road, Oakland, CA 94610 (US). QUARANTA, Vito ; 8861 Nottingham Place, La Jolla, CA 92037 (US). PYTELA, Robert ; 22 Flint Street, San Francisco, CA 94114 (US).

(74) Agents: CAMPBELL, Cathryn et al.; Pretty, Schroeder, Brueggemann & Clark, 444 South Flower Street, Suite 2000, Los Angeles, CA 90071 (US).

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(57) Abstract

The present invention provides substantially pure integrins containing a novel β subunit designated as β_6. The novel β_6 subunit forms heterodimers with α_V and α_F. Methods of controlling cell adhesion using the β_6-containing integrins are also provided.

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A NOVEL INTEGRIN β SUBUNIT AND USES THEREOF

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TECHNICAL FIELD

This invention relates to receptors for adhesion peptides, and more specifically to a novel receptor subunit having affinity for extracellular matrix molecules.

BACKGROUND ART

Multicellular organisms, such as man, have some 10^{14} cells which can be divided into a minimum of fifty different types, such as blood cells and nerve cells. During the course of growth and development, cells adhere to other cells, or to extracellular materials, in specific and orderly ways. Such cell adhesion mechanisms appear to be of importance in mediating patterns of cellular growth, migration and differentiation, whereby cells develop specialized characteristics so as to function as, for example, muscle cells or liver cells. Cell adhesion mechanisms are also implicated in dedifferentiation and invasion, notably where cells lose their specialized forms and become metastasizing cancer cells.

The mechanisms underlying the interactions of cells with one another and with extracellular matrices are not fully understood, but it is thought that they are mediated by cell surface receptors which specifically recognize and bind to a cognate ligand on the surface of cells or in the extracellular matrix.

The adhesion of cells to extracellular matrices and their migration on the matrices is mediated in many cases by the binding of a cell surface receptor to an Arg-Gly-Asp containing sequence in the matrix protein, as reviewed in Ruoslahti and Pierschbacher, Science 238:491 (1987). The Arg-Gly-Asp sequence is a cell attachment site at least in fibronectin, vitronectin, fibrinogen von Willibrand, thrombopondin, osteopontin, and possibly various collagens, laminin and tenascin. Despite the similarity of their cell attachment sites, these proteins can be recognized individually by their interactions with specific receptors.

The integrins are a large family of cell surface glycoproteins that mediate cell-to-cell and cell-to-matrix adhesion as described, for example, in the Ruoslahti and Pierschbacher article cited above. All known members of this family of adhesion receptors are heterodimers consisting of an α and a ß subunit noncovalently bound to each other. When the integrin family was first identified, integrins were grouped into three subfamilies based on the three ß subunits that were initially recognized ($ß_1$, $ß_2$ and $ß_3$). Over the past few years, the primary structures of three integrin ß subunits from mammalian cells and one from Drosophila have been deduced from cDNA.

Each α subunit was thought to associate uniquely with a single ß subunit. Eleven distinct α subunits have thus far been described. As new integrins have been identified, however, it has become clear that this grouping is not entirely satisfactory, since there are clearly more than three ß subunits and since some α subunits can associate with more than one ß subunit as described, for example, in Sonnenberg et al., J. Biol. Chem. 265:14030-14038 (1988).

Because of the importance of integrins in mediating critical aspects of both normal and abnormal cell processes, a need exists to identify and characterize different integrins. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention relates to a substantially purified β subunit of an integrin cell surface receptor designated as β_6. The amino acid sequence of β_6 is provided in Figure 3.

The present invention also relates to amino acid fragments specific to β_6 that have a variety of uses. The invention further relates to vectors having a gene encoding such fragments. Host cells containing such vectors are also provided. The nucleic acids encoding β_6 as well as nucleic acids that specifically hybridize with the nucleic acids encoding β_6 sequences are other aspects of the present invention.

In a further aspect, the present invention relates to a substantially purified integrin comprising β_6 bound to an α subunit, particularly α_V or α_F. Methods of blocking the attachment of the β_6-containing integrins to its ligand and of detecting the binding of such integrins to its ligand are also provided.

The present invention also relates to methods of increasing or decreasing cell adhesion in cells expressing a β_6-containing integrin by overexpressing the integrin or by binding the integrin with a ligand, such as vitronectin.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the design of PCR primers.

Figure 2 shows a map of sequencing strategy.

Figure 3 shows the nucleotide sequence and amino acid translation for human (H) and guinea pig (GP) β_6.

Figure 4 shows the alignment of β_6 with four previously reported integrin β subunits.

Figure 5 shows the alignment of partial nucleotide and amino acid sequences from human (H) and guinea pig (GP) β_1, β_2, β_3, and β_6 for the region just downstream from the B3F primer.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a composition of matter relating to a novel, substantially purified integrin β subunit, referred to herein as β_6. The amino acid sequence of β_6 is also provided and is shown in Figure 3.

By "substantially purified" is meant substantially free of contaminants normally associated with a native or natural environment.

By "β_6" is meant a polypeptide having substantially the same amino acid sequence and binding functions of the polypeptides encoded by the sequences set forth in Figure 3 for human and guinea pig β_6. Thus, modified amino acid sequences that do not substantially destroy the functions and retain the essential sequence of β_6 are included within the definition of β_6. Amino

acid sequences, such as the sequence for β_1, β_2 and β_3, having less than 50% homology with the sequence of β_6, are not substantially the same sequence and, therefore, do not fall within the definition of β_6. Given the amino acid sequences set forth herein, additions, deletions or substitutions can be made and tested to determine their effect on the function of β_6. In addition, one skilled in the art would recognize that certain amino acids, such as the conserved cystines, for example, can be modified to alter a binding function of β_6.

Amino acids are identified herein by the standard one-letter abbreviations, as follows:

Amino Acid	Symbol
Alanine	A
Asparagine	N
Aspartic acid	D
Arginine	R
Cysteine	C
Glutamine	Q
Glutamic acid	E
Glycine	G
Histidine	H
Isoleucine	I
Leucine	L
Lysine	K
Methionine	M
Phenylalanine	F
Proline	P
Serine	S
Threonine	T
Tryptophan	W
Tyrosine	Y
Valine	V

Based on its amino acid sequence, the β subunit of the present invention is clearly different from β_1, β_2, β_3 and other β subunits that have recently been discovered. For example, the 11-amino acid carboxyl-terminal extension on β_6 distinguishes it from β_1, β_2, and

β_3. The short cytoplasmic tails of β_1, β_2, and β_3 are thought to be sites of interaction with the cytoskeleton and regions for the transduction of signals initiated by interactions of the large extracellular domains with ligands. These cytoplasmic tails may also be targets for regulation of integrin function. The distinctive 11-amino acid cytoplasmic tail of β_6 indicates that its regulation or pathways for signal transduction may be different from those of β_1, β_2 and β_3.

In addition to β_1, β_2 and β_3, recent studies have suggested the existence of as many as five other integrin β subunits. A β subunit with a molecular weight of approximately 210,000 (β_4) has been found associated with the integrin α subunit "α_6" in colon carcinoma cells and in a variety of other tumor cells of epithelial origin as described, for example, in Kajiji et al., EMBO J., 8:673-680 (1989). On the basis of its high molecular weight, 210,000 compared with the predicted size of 106,000 of the subject novel protein, and on the basis of its clearly different amino-terminal sequence, it is apparent that β_4 is not the same as the subject polypeptide.

Another β subunit, originally called β_x was identified in epithelial-derived tumor cells in association with the integrin α subunit α_v as described, for example, in Cheresh et al., Cell 57:59-69 (1989). This β subunit, having a distinctive amino-terminal sequence, was recently renamed β_5. Based on recent studies of purified preparations, β_5 clearly differs from the β subunit of the present invention. Because the β subunit described in the present report is distinct from each of the five β subunits for which sequence information is available, it has been designated as β_6.

The existence of two other integrin β subunits

has been inferred from the identification of unique proteins after immunoprecipitation of surface-labeled cell lysates with antibodies to known α subunits. One of these novel proteins, called β_s was found in association with α_v in the human osteosarcoma cell line MG-63, in the fibroblast cell line AF1523, and in human endothelial cells as described, for example, in Freed et al., EMBO J. 8:2955-2965 (1989). This subunit is also different from β_6 since β_s is expressed in MG-63 cells while β_6 is not expressed in these cells as shown in Table 1.

The other novel integrin β subunit identified by co-immunoprecipitation of known α subunits, β_p, is a protein of about M_r 95,000 that is found to be associated with α_4, an α subunit first found as part of the lymphocyte homing receptor VLA-4 as described, for example, in Holzmann et al., Cell 45:37-46 (1989). This subunit is also distinct from β_6 since β_p is expressed in lymphocytes while β_6 is not expressed in lymphocytes as shown in Table 1.

TABLE 1

Distribution of β_6

	Type	Results	Source
Cell Lines:			
FG-2	Pancreatic	+	Kajiji et al. EMBO J 3:673-80 (1989)
Panc I	Pancreatic	-	Dr. Metzgar, Duke U., N.C.
Colo-396	Colon CA	+	Dr. L. Walker, Cytel, San Diego, CA
UCLA P3	Lung CA	+	Dr. L. Walker, Cytel, San Diego, CA
Hela	Cervical	-	ATCC #CCL-2
Jar	Chorio CA	+	ATCC #HTB 36
HT 1080	Fibrosarcoma	-	ATCC #CCL 121
U 937	Monocytoid	-	ATCC #CRL 1593
M 21	Melanoma	-	Dr. R. Reisfeld, Scripps Clinic & Research Foundation, La Jolla, CA
B 16	Melanoma	-	Dr. R. Reisfeld Scripps Clinic & Research Foundation, La Jolla, CA
MG 63	Osteosarcoma	-	ATCC #CRL 1427
Tissues:			
	Cervix	+	
	Aortic Endothelium	-	
	Leukocytes	-	

The invention also provides an integrin comprising β_6 bound to an α subunit. β_6, consistent with recent findings of other β subunits, can associate with a variety of α subunits to form a functional integrin. In one embodiment, β_6 associates with α_v. In another embodiment, β_6 associates with another α subunit referred to herein as α_F. The α_v β_6 integrin, as well as other integrins containing β_6, can bind molecules, for example extracellular matrix molecules. Such molecules are referred to herein as ligands. In a specific embodiment, certain β_6-containing integrins can bind Arg-Gly-Asp-containing polypeptides such as vitronectin or fibronectin. The binding of β_6-containing integrins to various ligands can be determined according to procedures known in the art and as described for example, in Ruoslahti & Pierschbacher, Science 238:491-497 (1987).

The invention also provides an amino acid fragment specific to β_6. Since β_6 is a novel molecule, it contains many fragments which are specific for this β subunit. Fragments specific to β_6 contain sequences having less than 50% homology with sequences of other known integrin β subunit fragments. These fragments are necessarily of sufficient length to be distinguishable from known fragments and, therefore, are "specific for β_6." The amino acid sequence of such fragments can readily be determined by referring to the figures which identify the β_6 amino acid sequences. These fragments also retain the binding function of the β_6 subunit and can therefore be used, for example, as immunogens to prepare reagents specific for β_6 or as an indicator to detect the novel β_6-containing integrin of the present invention. One skilled in the art would know of other uses for such fragments.

The invention also provides a reagent having specificity for an amino acid sequence specific for β_6.

Since β_6 is a novel protein with at least 50% amino acid differences over related β-subunits, one skilled in the art could readily make reagents, such as antibodies, which are specifically reactive with amino acid sequences specific for β_6 and thereby immunologically distinguish β_6 from other molecules. Various methods of making such antibodies are well established and are described, for example, in Antibodies, A Laboratory Manual, E. Harlow and D. Lane, Cold Spring Harbor Laboratory 1988, pp. 139-283 and Huse et al., Science 24:1275-1280 (1988).

The invention also provides nucleic acids which encode β_6. Examples of such sequences are set forth in Figure 3. Following standard methods as described, for example, in Maniatis et al., Molecular Cloning, Cold Spring Harbor (1982), nucleic acid sequences can be cloned into the appropriate expression vector. The vector can then be inserted into a host, which will then be capable of expressing recombinant proteins. Thus, the invention also relates to vectors containing nucleic acids encoding such sequences and to hosts containing these vectors.

The sequences set forth in Figure 3 also provide nucleic acids that can be used as probes for diagnostic purposes. Such nucleic acids can hybridize with a nucleic acid having a nucleotide sequence specific for β_6 but do not hybridize with nucleic acids encoding non-β_6 proteins, particularly other cell surface receptors. These nucleic acids can readily be determined from the sequence of β_6 and synthesized using a standard nucleic acid synthesizer. Nucleic acids are also provided which specifically hybridize to either the coding pr non-coding DNA of β_6.

Integrin cell surface receptors bind ligands, such as extracellular matrix molecules. However, the

binding of the integrin to the ligand can be blocked by various means. For example, the binding of a β_6-containing integrin can be blocked by a reagent that binds the β_6 subunit or the β_6-containing integrin. Examples of such reagents include, for example, Arg-Gly-Asp-containing peptides and polypeptides, ligand fragments containing the integrin binding site, as well as antibodies specifically reactive with β_6 or a β_6-containing integrin. Alternatively, the blocking can be carried out by binding the ligand or fragment thereof, recognized by a β_6-containing integrin with a reagent specific for the ligand at a site that inhibits the ligand from binding with the integrin. Since the binding of a β_6-containing integrin to its ligand can mediate cell adhesion to an extracellular matrix molecule, preventing this binding can prevent cell adhesion. Alternatively, cell adhesion can be promoted by increasing the expression of β_6-containing integrins by a cell.

Finally, the invention provides a method of detecting ligands which bind a β_6-containing integrin. The method comprises contacting a β_6-containing integrin with a solution containing ligands suspected of binding β_6-containing integrins. The presence of ligands which bind a β_6-containing integrin is then detected.

In summary, the invention claims:

1. A substantially purified integrin cell surface receptor subunit comprising β_6.

2. The substantially purified integrin cell surface receptor subunit of claim 1 having the amino acid sequence set forth in Figure 3 for human.

3. A substantially purified integrin comprising β_6 bound to an α subunit.

4. The integrin of claim 3, wherein the subunit is α_v.

5. The integrin of claim 3, wherein the subunit is α_F.

6. A substantially purified amino acid fragment specific to β_6.

7. A vector comprising a gene encoding for the amino acid fragment of claim 6.

8. A host containing the vector of claim 7.

9. A reagent having specificity for an amino acid sequence specific for β_6.

10. The reagent of claim 9, wherein the reagent is an antibody.

11. A substantially purified nucleic acid encoding β_6.

12. A substantially purified nucleic acid which specifically hybridizes with a nucleotide sequence of the nucleic acid of claim 11.

13. A substantially purified nucleic acid which specifically hybridizes with the nucleic acid of claim 12 and does not hybridize with a nucleic acid encoding a non-β_6 polypeptide.

14. A method of preventing the binding of a cell expressing a β_6-containing integrin to ligand capable of binding to said β_6-containing integrin, comprising blocking the binding of the β_6-containing integrin and the ligand.

15. The method of claim 14, wherein the blocking is effected by binding the β_6-containing integrin with a reagent specific thereto.

16. The method of claim 14, wherein the blocking is effected by binding the ligand of the β_6-containing integrin with a reagent specific for the ligand.

17. The method of claim 15, wherein the reagent is an RGD-containing peptide or polypeptide.

18. The method of claim 15, wherein the reagent is a ligand fragment containing an integrin binding site.

19. A method of detecting a ligand that binds a β_6-containing integrin, comprising contacting the β_6-containing integrin with a solution containing the ligand suspected of binding β_6-containing integrins and detecting the presence of the ligand bound to the β_6-containing integrin.

20. A method of increasing cell adhesion in cells expressing a β_6-containing integrin, comprising overexpressing the β_6-containing integrin in a cell.

21. A method of decreasing cell adhesion in cells expressing a β_6-containing integrin comprising binding the β_6-containing integrin with a ligand.

The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

Identification of a Novel ß Subunit

Generation of cDNA Fragments by Polymerase Chain Reaction

Tracheal epithelial cells harvested from male Hartley outbred guinea pigs (Charles River Breeding Laboratories, Bar Harbor, ME) were grown to confluence over 10-14 days on collagen-impregnated microporous filters commercially available from Costar. RNA was harvested from these primary cultures, and mRNA was purified over oligo(dT)-cellulose columns using the Fast Track mRNA isolation kit (Invitrogen, San Diego, California). Two to 5 μg of mRNA was used as a template for cDNA synthesis catalyzed by 200 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) in a 20-40 μl reaction volume. One to 5 μl of the resultant cDNA was used as a template for polymerase chain reaction (PCR). PCR was carried out in a reaction volume of 25-200 μl. In addition to the template cDNA, each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM each of dATP, dGTP, dCTP and dTTP, and 0.05 units/μl Taq DNA polymerase (obtained from either United States Biochemical Corporation, Cleveland, OH, or from Promega, Madison, WI).

For each reaction, two oligonucleotide primers were also added to obtain a final concentration of 1 μM each. The primer pairs are identified below. Each reaction mixture was overlaid with mineral oil, heated to 95°C for 4 min. in a thermal cycler (Ericomp, San Diego, CA), and then subjected to 30 cycles of PCR. Each cycle

consisted of 45 seconds at 95°C, 45 seconds at 53°C, and 1 min. at 72°C. Immediately after the last cycle, the sample was maintained at 72°C for 10 min.

The results of each PCR reaction were analyzed by gel electrophoresis in 1.5% agarose. Reactions that produced fragments of the expected size were electrophoresed in 1.5% low gel temperature agarose (Bio-Rad Laboratories, Richmond, CA). The appropriate size band was excised, melted at 68°C, and the DNA was purified by extraction with phenol/chloroform and precipitation in ethanol and ammonium acetate.

PCR Primers

To obtain the initial fragment of the novel ß subunit cDNA described herein, degenerate mixtures of PCR primers were used. Oligonucleotides were synthesized, trityl-on, by the University of California, San Francisco Biomolecular Resource Center using a DNA synthesizer with standard procedures, and purified over Nen-sorb cartridges (DuPont-New England Nuclear, Boston, MA). These consensus primer mixtures were designed to anneal with the nucleotides encoding the highly conserved sequence Asp-Leu-Tyr-Tyr-Leu-Met-Asp-Leu (primer B1F) and Glu-Gly-Gly-Asp-Ala-Ile-Met-Gln (primer B2R) that flank an approximately 300-nucleotide region beginning approximately 130 amino acids from the amino terminus of each of the integrin ß subunits sequenced to date. The sequences of the primers identified herein are depicted in Figure 1.

On the basis of the initial sequence obtained, a specific forward primer was designed to anneal with the sequence encoding the amino acids Pro-Leu-Thr-Asn-Asp-Ala-Glu-Arg (primer BTE2F) ending approximately 49

nucleotides from the 3' end of the region we had sequenced. We also designed an additional forward primer (B3F) and two reverse primers (B3R and B4R) to recognize highly conserved consensus regions encoding the sequences Gly-Glu-Cys-Val-Cys-Gly-Gln-Cys (B3 region) and Ile-Gly-Leu-Ala-Leu-Leu-Leu-Ile-Trp-Lys (B4 region). The alignment of these primers with previously published sequences of human β_1, β_2 and β_3 and chicken β_1 is shown in Fig. 1. PCR as described above was performed with cDNA from guinea pig tracheal epithelial cells and the primer pairs BTE2F/B3R and B3F/B4R.

The primer pair BTE2F/B3R yielded 1095 additional base pairs of new sequence. Based on this sequence another specific primer (BTE3F) was designed to recognize the sequence Val-Ser-Glu-Asp-Gly-Val near the 3' end of this sequence, and PCR was performed with this primer in combination with primer B4R.

Figure 1 shows the design of PCR primers. β subunit consensus primer mixtures were designed on the basis of alignment of published sequences of human β_1, β_2, β_3 and chicken β_1. For forward primers (B1F and B3F), the primer sequences included a single nucleotide whenever possible for each of the first two nucleotides of each codon and were usually either degenerate or included deoxyinosine for the third base in codons for amino acids other than methionine. Reverse primers (B2R, B3R, and B4R) were designed in the same manner for the complementary DNA strand. Two specific forward primers were designed to recognize β_6. The first (BTE2F) was designed to work across species and was thus degenerate or included deoxyinosine in the third codon position. The second, BTE3F, was not degenerate and was designed to only recognize guinea pig β_6.

Cloning of Fragments Obtained by PCR

Individual fragments were cloned in pBluescript (Stratagene, San Diego, CA) as follows. Purified fragments were resuspended in distilled water containing deoxynucleotides and treated with 2.5 units of DNA polymerase I, large fragment (Promega) to fill in any 3' recessed ends left after the last cycle of PCR. The 5' ends were phosphorylated with 5 units of T4 polynucleotide kinase (New England Biolabs, Beverly, MA). An aliquot of the above reaction mixture containing approximately 100-200 ng of DNA, was ligated into pBluescript that had been cut with EcoRV (Promega) and dephosphorylated with calf intestinal alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN). Ligations were performed at 22°C for 1 hour with T4 DNA ligase (Bethesda Research Laboratories). The ligation mixture was used to transform competent Escherichia coli (JM109, Clontech, San Francisco, California). Plasmids containing inserts were purified using the Pharmacia miniprep lysis kit (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) denatured in 0.3 M NaOH, further purified over spin columns containing Sephacryl S-400 (Pharmacia), and then sequenced using the Sequenase™ version 2.0 sequencing kit (United States Biochemical Corp., Cleveland, OH) and [^{35}S]dATP (Amersham Corp., Arlington Heights, IL).

Library Screening

PCR fragments generated with the primer pairs B1F/B2R and BTE3F/B4R were uniformly labeled with alpha-[^{32}P]dCTP and used as probes to screen a random-primed cDNA library and an oligo-dT-primed cDNA library both constructed in the plasmid pTZ18R-BstXI (Invitrogen) from mRNA obtained from the human pancreatic carcinoma cell line FG-2. Plasmid was purified from clones found to

hybridize with either region, and inserts were sequenced. A portion of insert DNA from one clone was in turn labeled and used to screen the same libraries. Fourteen independent overlapping clones were sequenced from both ends using primers that recognize regions of the pTZ polylinker. The regions flanking the 3' end of the putative translated region of the new ß subunit were sequenced in both directions from three clones using primers constructed to recognize sequences close to the 3' end. On the basis of the initial sequences thus obtained, an additional internal sequence was obtained from clones T10, T11, T12 and T14 (Fig. 2) after digestion with specific restriction endonucleases and relegation. Three internal fragments thus generated were subcloned into pBluescript and were also sequenced in both directions. Approximately 90% of the new sequence reported was obtained from both strands of DNA, and 97% was obtained from two or more overlapping clones (Fig. 2).

Figure 2 shows a map of the sequencing strategy. Shown are the location of clones used to obtain the partial cDNA sequence of guinea pig $ß_6$ (clones 1F, 3L, 3N and 3Y, top) and the complete sequence of human $ß_6$ (clones T1-T19 bottom). Also shown is the location of the translated region (Protein). The location of the transmembrane domain is shown by the letters TM. Clones shown often represent one of several identical clones. Internal sequence of clones with long inserts was obtained by restriction endonuclease digestion and relegation and by ligation of internal fragments into pBluescript. Specific restriction sites employed are shown (Hind, HindIII; Hinc, HincII; Kpn, KpnI; Pst, PstI). The direction and extent of sequencing are shown by arrows. 1109 and 1110 are the sites recognized by oligonucleotide sequencing primers. T18 and T19 each terminated in a poly(A) tail. The regions

recognized by the degenerate PCR primers B1F (B1), B2R (B2), B3R/F (B3)., and B4R (B4) and the β_6 primers BTE2F (BTE2) and BTE3F (BTE3) are noted above the guinea pig cDNA map, kb, kilobases.

Nucleotide Sequence of a Novel Guinea Pig Integrin ß Subunit

PCR using cDNA from guinea pig airway epithelial cells and the consensus primer mixtures B1F and B2R (Fig. 1) amplified DNA fragments with the expected size of approximately 350 nucleotides. When the fragment DNA was sequenced after cloning into pBluescript, recombinant clones each contained inserts with one of two distinct sequences. One sequence encoded a stretch of 98 amino acids that was 97% identical to the expected region of human β_1 and was therefore presumed to be guinea pig β_1. The other sequence encoded 98 amino acids that were only 53% identical to human β_1, 45% identical to human β_2, and 57% identical to human β_3 (Fig. 2, clone 1F). Both of the guinea pig sequences included the integrin ß subunit consensus sequences Ser-X-Ser-Met-X-Asp-Asp-Leu and Gly-Phe-Gly-Ser-Phe-Val, and both contained the 2 cysteine residues found in this region in all known integrin ß subunits. These data suggest that one of the two sequences we obtained encoded a new member of the integrin ß subunit family.

This novel sequence was extended by further PCR steps utilizing primers specific for the novel sequence (BTE2F, BTE3F) in combination with two additional degenerate primers (B3R and B4R, see Figs. 1, 2 and 4). With the primer pair BTE2F/B3R two different cDNA products were obtained (3L and 3N in Fig. 2) due to an unexpected hybridization of the B3R primer with a site 220 nucleotides further downstream (B3' in Fig. 2). The 1732-nucleotide sequence determined from these clones is

shown in Fig. 3.

Figure 3 shows Nucleotide sequence and amino acid translation for human (H) and guinea pig (GP) β_6. The amino acid translation is denoted by the single letter code beneath the second nucleotide of each codon from the translated region of human β_6. For the guinea pig sequence, only amino acids that differ from the human sequence are shown. The numbers along the right-hand margin denote the nucleotide or amino acid number of the last entry on each line. The numbering system used starts with the first nucleotide or amino acid available for each sequence shown. The nine potential sites for N-glycosylation in the putative extracellular domain of human β_6 are underlined.

Nucleotide Sequence of Human β_6

Screening of cDNA libraries constructed from the human pancreatic carcinoma cell line FG-2 with guinea pig cDNA probes 1F and 3Y (see Fig. 2) and subsequent screening with a probe constructed from a portion of clone T10 (Fig. 2) produced 14 independent positive clones. The two longest clones (T18 and T19) extended to the poly(A) tail. A map of these clones, constructed on the basis of sequence information and of the mobility of inserts cut out of these clones in agarose gels is shown in Fig. 2. This map predicts an mRNA of approximately 5 kilobases including at least a 226-nucleotide untranslated region at the 5' end and, a 2364-nucleotide open reading frame, and a 3' untranslated region of approximately 2.5 kilobases. This molecule has been termed integrin β_6.

Fig. 3 shows the partial nucleotide and complete amino acid sequences for human β_6 (excluding most of the 3'-untranslated region) and the alignment of the

1732 nucleotides of sequence obtained from PCR of guinea pig airway epithelial cell cDNA. Of the 577 amino acids deduced from the region sequenced in both species only 36 residues differ; the amino acid sequences are 94% identical. Furthermore, of the 1732 nucleotides sequenced in both species, 91% are identical. Nine potential glycosylation sites present in the putative extracellular domain of human β_6 are shown by underlining. All seven of these sites that lie within the 577 amino acids obtained for guinea pig β_6 are also present in the guinea pig protein. If all of the potential glycosylation sites are occupied with oligosaccharides having an average molecular weight of 2,500, the predicted molecular weight of human β_6 would be 106,000.

Comparison of the 788-amino acid sequence deduced from the open reading frame to the three previously sequenced human β subunits and the myospheroid protein of Drosophila is shown in Fig. 4.

Figure 4 shows the alignment of β_6 with four previously reported integrin β subunits. Previously published sequences for human β_1, human β_2, human β_3, the myospheroid gene product (βmyo) of Drosophila, and the novel sequence described as (β_6) are shown using the single letter amino acid code. The 56 conserved cysteines are noted by * and the 120 other invariant amino acids by = above each line. The transmembrane domain is underlined. The regions used for constructing the consensus β subunit primers B1F (1), B2R (B2), B3F/R (B3), and B4R (B4) are labeled below the alignment in bold type. The numbers along the right-hand margin denote the number of the last amino acid in each line beginning from the first amino acid of each putative signal sequence.

There are 179 amino acid residues that are identical in each of the other ß subunits and in $ß_6$ including 56 conserved cysteine residues. The overall percentage of identical amino acids between $ß_6$ and the other human ß subunits is 47% for $ß_3$, 42% for $ß_1$ and 38% for $ß_2$. Human $ß_6$ is also 39% identical to the Drosophila ß subunit. Human $ß_1$, $ß_2$ and $ß_3$ and the Drosophila ß subunit all have cytoplasmic regions consisting of 41 amino acids (beginning after the putative transmembrane domain shown by the underline in Fig. 4). Although $ß_6$ contains each of the 10 conserved amino acid residues in this cytoplasmic region it also contains an 11-amino acid extension at the carboxyl terminus. $ß_6$ also contains two Arg-Gly-Asp sequences, one at amino acids 514-516 and the other at 594-596. These regions could serve as recognition sites for other ligands of the integrin family.

PCR using the primer pair B3F/B4R (see Fig. 1) amplified fragments of the expected size of approximately 750 nucleotides. Cloning and sequencing of the fragments did not result in any additional clones containing the novel ß subunit sequence but did result in several clones with inserts encoding an amino acid sequence that was 97% identical to the corresponding region of human $ß_3$ and several others encoding an amino acid sequence that was 93% identical to human $ß_1$ (Fig. 5). These are presumably the guinea pig homologues of $ß_1$ and $ß_3$, respectively. The nucleotide sequences of guinea pig and human $ß_1$ are 80% identical, and those of guinea pig and human $ß_3$ are 91% identical.

Figure 5 shows the alignment of partial nucleotide and amino acid sequences from Human (H) and guinea pig (GP) $ß_1$, $ß_2$, $ß_3$, and $ß_6$ for the region just downstream from the B3F primer. Amino acid translations denoted by the one-letter code are shown below the second

nucleotide of each codon. For the guinea pig sequences, only amino acids that differ from the human sequences are shown. The numbers shown along the right-hand margin denote the nucleotide number for human β_6. The sequences for human β_1 and β_3 are from previously published reports.

EXAMPLE II

β_6 Associates with α_v And α_f Subunits

To determine that the novel β subunit of the present invention is associated with an α chain similar to other known integrins, antisera against peptides from the cytoplasmic domain sequence of β_6 were prepared. The following amino acid peptides from the cytoplasmic sequence of β_6 were prepared and used to immunize rabbits: RGSTSTFKNVTYKHR (residues 763-777) and YKHREKQKVDLSTDC (residues 774-788). The antisera were raised in rabbits according to standard procedures known in the art. Briefly, peptides were chemically coupled to keyhole lympet hemocyanin, and were injected in rabbits in either complete (first injection only) or incomplete Freund's adjuvant as described, for example, in Antibodies: A Laboratory Manual, E. Harlow and D. Lowe, eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724. Antisera were termed 6830 (to peptides corresponding to residues 763-777) and 6341 (to peptides corresponding to residues 774-788).

The resulting polyclonal antibodies were used to immunoprecipitate detergent lysates from the pancreatic carcinoma cell line FG-2 that had been surface radioiodinated according to procedures well known in the art such as described, for example, in Kajiji et al., EMBO J 3:673-680 (1989). A complex of two bands was precipitated of respectively 150 kilodaltons (Kd) and 97 Kd in SDS-PAGE under non-reducing conditions. Under reducing conditions, the two bands migrated as a diffused

band, extending from 130 Kd to 116 Kd. These bands were specific since pre-immune serum did not precipitate any of them and they were not present when the immunoprecipitation was carried out in the presence of the corresponding immunogenic peptide. Furthermore, the same complex of two bands was precipitated by both the 6830 and 6841 antibodies, which were raised against independent peptides from the cytoplasmic sequence deduced from β_6 cDNA clones.

To determine which of the two precipitated bands corresponds to β_6, a SDS-heat denaturated lysate from surface-radioiodinated FG-2 cells was immunoprecipitated with the 6841 antibody. Only the 97 Kd band was detectable (non-reducing conditions), identifying it as the β_6 band. Under reducing conditions, the apparent molecular weight of this band increased to 116 Kd suggesting the presence of many intra-chain disulfide bonds, which is consistent with the primary structure of β_6 and of other integrin β chains.

The other band, of 150 Kd or 130 Kd under non-reducing or reducing conditions, respectively, is likely to be an α subunit since it dissociates after SDS-heat denaturation of the lysate, indicating that it is non-covalently associated with the β_6 polypeptide. Furthermore, similar to certain other integrin α chains, its molecular weight decreases under reducing conditions by about 20 Kd (130 Kd versus 150 Kd under non-reducing conditions) probably due to a disulfide linked small peptide that dissociates upon reduction.

To identify which α chain is associated with β_6, the $\alpha\beta_6$ integrin complex was purified by immuno-affinity chromatography on a 6841-protein A sepharose matrix according to procedures well known in the art such as described, for example, in Kajiji et al., EMBO J 3:673-

680 (1989). The eluted material was immunoprecipitated with antibodies specific for α_1, α_2, α_3, α_5, α_6 and α_v, which are known to be expressed in FG-2 cells. Only the anti-α_v monoclonal antibody 142.19, obtained from David Cherish, Ph.D., Scripps Clinic and Research Foundation, La Jolla, California, reacted with the purified material, which indicates that the α_v is associated with β_6 in this pancreatic carcinoma cell line.

To confirm this data, immunodepletion experiments on surface-radioiodinated FG-2 lysates were performed according to methods well known in the art such as described in Kajiji et al., EMBO J 3:673-680 (1989). The cell lysate was depleted with the 6841 anti-β_6 antibody or, in parallel, with a control antiserum, and then immunoprecipitated with the 142.19 anti-α_v antibody. A smaller amount of α_v was present in the immunoprecipitation on the β_6 depleted lysate and no 97 Kd β_6 band was visible. Instead, a smaller band of about 90 Kd was present. It is hypothesized that this smaller band represents the β_5 chain also associated with α_v in these cells. In the control lysate depleted with normal rabbit serum, all three bands, 150 Kd (α_v), 97 Kd (β_6) and 90 Kd (β_5) were present after immunoprecipitation with the anti-α_v 142.19 antibody.

Another immunodepletion was carried out using 142.19 antibody as the depleting antibody, or in parallel a mouse monoclonal as a control antibody. Immunoprecipitations of α_v-depleted lysate with anti-α_v 142.19 antibodies did not show the presence of any band, indicating that all α_v-containing integrins had been removed. However, the 6841 anti-β_6 antibody still precipitated a complex of two bands, one corresponding to β_6, the other with a molecular weight close to that of α_v. This α chain, however, must differ from α_v since it is unreactive with anti-α_v monoclonal antibodies and is

referred to herein as α_F. In the control depleted lysates, the 6841 anti-β_6 antibody precipitates much stronger bands, consistent with the possibility that, in FG-2 cells, two β_6 integrins exist, $\alpha_V\beta_6$ and $\alpha_F\beta_6$.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

WE CLAIM:

1. A substantially purified integrin cell surface receptor subunit comprising β_6.

2. The substantially purified integrin cell surface receptor subunit of claim 1 having the amino acid sequence set forth in Figure 3 for human.

3. A substantially purified integrin comprising β_6 bound to an α subunit.

4. The integrin of claim 3, wherein the subunit is α_v.

5. The integrin of claim 3, wherein the subunit is α_f.

6. A substantially purified amino acid fragment specific to β_6.

7. A vector comprising a gene encoding for the amino acid fragment of claim 6.

8. A host containing the vector of claim 7.

9. A reagent having specificity for an amino acid sequence specific for β_6.

10. The reagent of claim 9, wherein the reagent is an antibody.

11. A substantially purified nucleic acid encoding β_6.

12. A substantially purified nucleic acid which specifically hybridizes with a nucleotide sequence of the nucleic acid of claim 11.

13. A substantially purified nucleic acid which specifically hybridizes with the nucleic acid of claim 12 and does not hybridize with a nucleic acid encoding a non-β_6 polypeptide.

14. A method of preventing the binding of a cell expressing a β_6-containing integrin to ligand capable of binding to said β_6-containing integrin, comprising blocking the binding of the β_6-containing integrin and the ligand.

15. The method of claim 14, wherein the blocking is effected by binding the β_6-containing integrin with a reagent specific thereto.

16. The method of claim 14, wherein the blocking is effected by binding the ligand of the β_6-containing integrin with a reagent specific for the ligand.

17. The method of claim 15, wherein the reagent is an RGD-containing peptide or polypeptide.

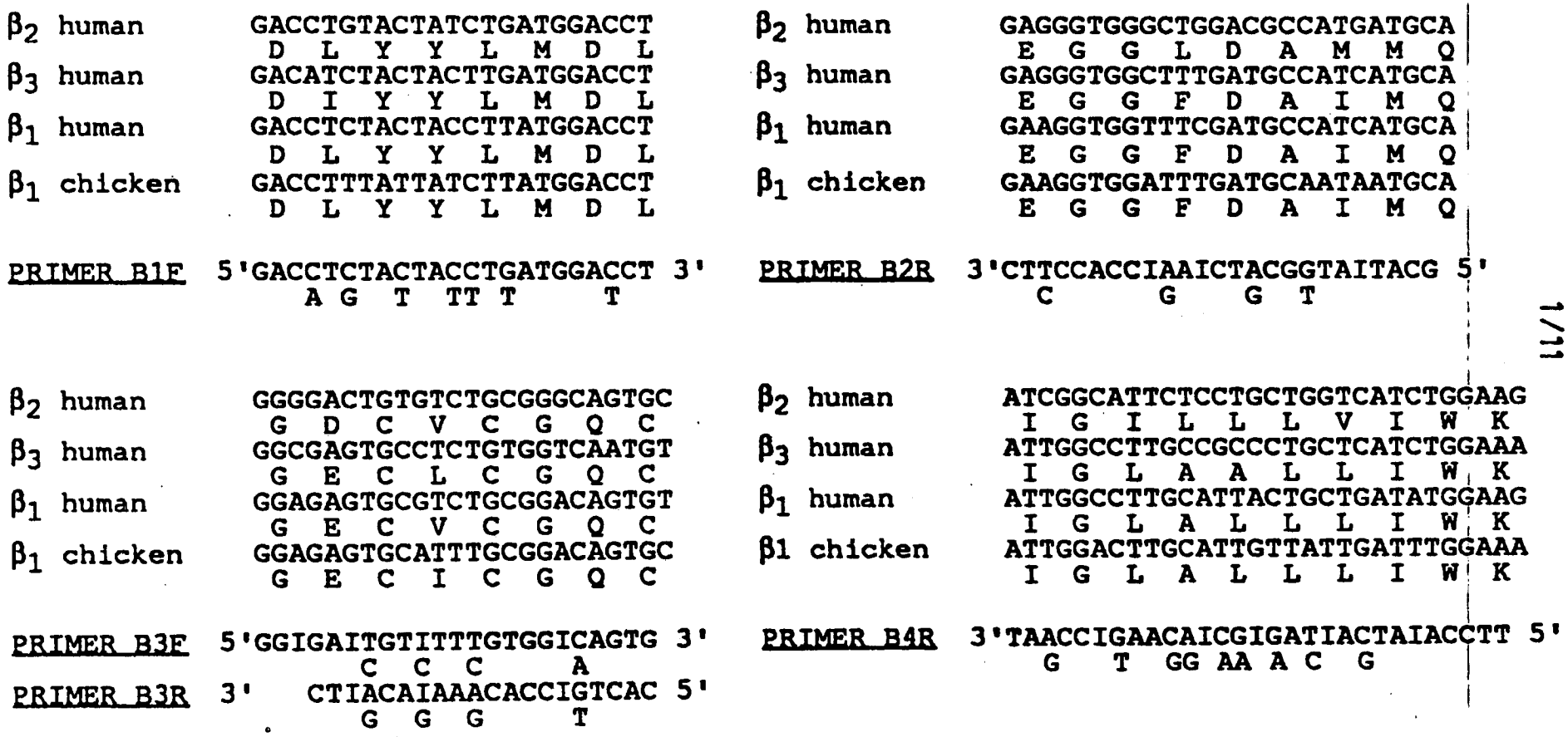
18. The method of claim 15, wherein the reagent is a ligand fragment containing an integrin binding site.

19. A method of detecting a ligand that binds a β_6-containing integrin, comprising contacting the β_6-containing integrin with a solution containing the ligand suspected of binding β_6-containing integrins and detecting the presence of the ligand bound to the β_6-containing integrin.

20. A method of increasing cell adhesion in cells expressing a β_6-containing integrin, comprising overexpressing the β_6-containing integrin in a cell.

21. A method of decreasing cell adhesion in cells expressing a β_6-containing integrin comprising binding the β_6-containing integrin with a ligand.

FIG. 1A CONSENSUS β SUBUNIT PRIMERS

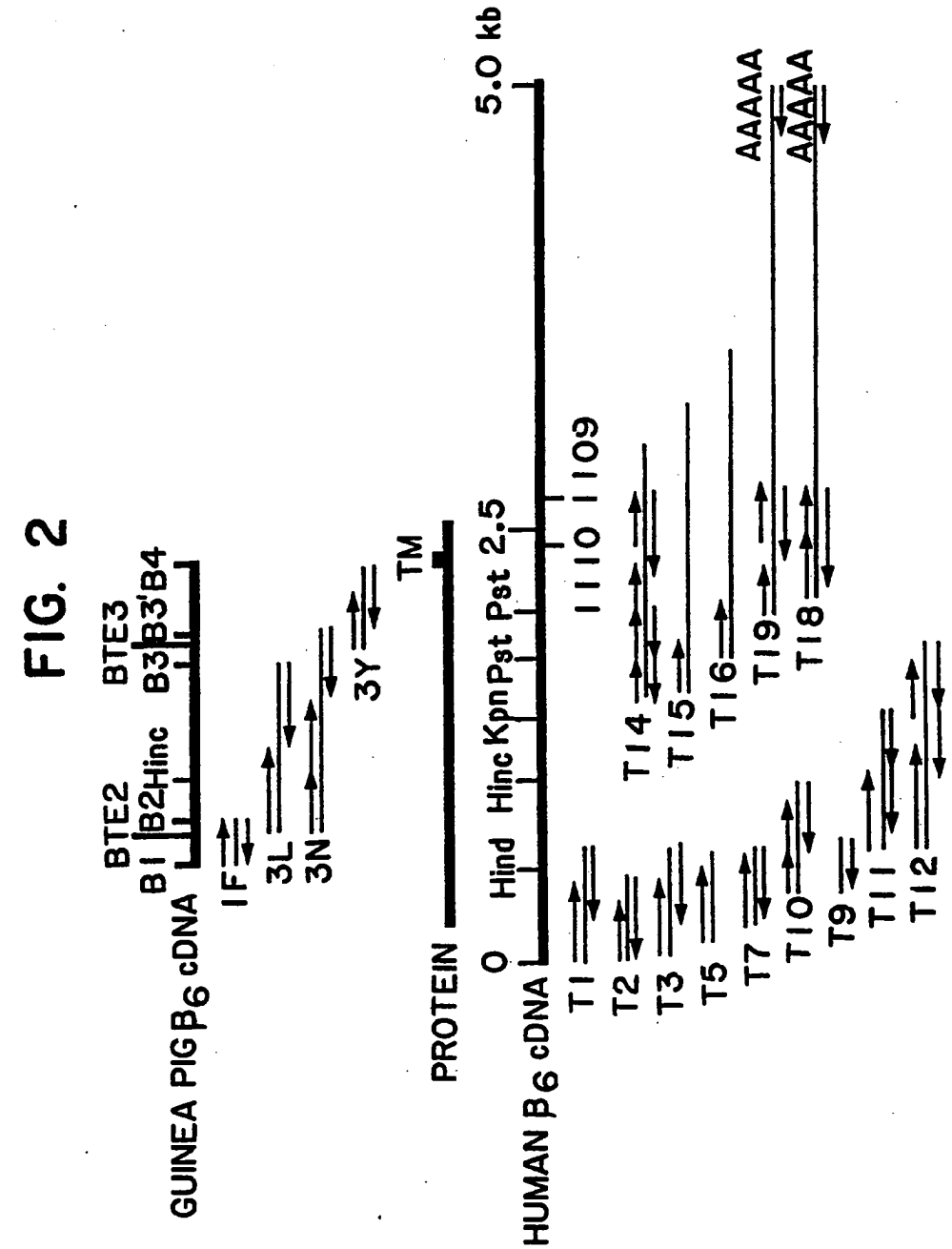


β_6 PRIMERS

	nt	
β_6 guinea pig	219	CCATTGACAAATGATGCTGAAAGA P L T N D A E R
PRIMER BTE2F		5'CCITTIACIAATGATGCIGAAAGA 3' C C
β_6 guinea pig	1325	CATCTCCGAAGACGGCA I S E D G
PRIMER BTE3F		5'CATCTCCGAAGACGGCA 3'

FIG. 1B

FIG. 2


GUINEA PIG β6 cDNA
BTE2
B1
B2
Hinc
BTE3
B3
B3'
B4
IF
3L
3N
3Y
TM
PROTEIN
0
Hind
Hinc
Kpn
Pst
Pst
2.5
5.0 kb
HUMAN β6 cDNA
T1
T2
T3
T5
T7
T10
T9
T11
T12
T14
T15
T16
T19
T18
AAAAA
AAAAA

```
β6HUMAN  TAAACACAGCTTTTCTGCTTTACCTGTCCAGGTAGCCTCTGTTTTCATT

β6H      AGTGTAAGTAGTATTTAAAATGTTATACTTCAAGAAAGAAAGACTTTAACG

β6H      CTCGCACAGCAAGAACTGAAACGAATGGGGATTGAACTGCTTTGCCTGTTC
                                    M  G  I  E  L  L  C  L  F
β6H      GGTGCAGAAACCTGTGAAGACTGCCTGCTTATTGGACCTCAGTGTGCCTGG
          G  A  E  T  C  E  D  C  L  L  I  G  P  Q  C  A  W
β6H      ACCCCAGCAAACCTTTTAGCTAAAGGATGTCAATTAAACTTCATCGAAAAC
          T  P  A  N  L  L  A  K  G  C  Q  L  N  F  I  E  N
β6H      CAGAAAAATAGTTCTGACATTGTTCAGATTGCACCTCAAAGCTTGATCCTT
          Q  K  N  S  S  D  I  V  Q  I  A  P  Q  S  L  I  L
β6H      GAGGACTACCCGGTGGATTTGTATTACCTCATGGACCTCTCCGCCTCCATG
          E  D  Y  P  V  D  L  Y  Y  L  M  D  L  S  A  S  M
                                    β6GUINEA PIG  TCCGCCTCCATG

β6H      ATGTCTAAATTAACCAGCAACTTTAGACTGGGCTTCGGATCTTTTGTGGAA
          M  S  K  L  T  S  N  F  R  L  G  F  G  S  F  V  E
β6GP     ATGTCTAAATTAACTAGCAACTTTAGACTGGGCTTCGGCTCTTTTGTAGAA

β6H      TGCAGTAGTATTCCATACTTCTGTTTACCTACATTTGGATTCAAGCACATT
          C  S  S  I  P  Y  F  C  L  P  T  F  G  F  K  H  I
β6GP     TGCAGTAGTATTCCATATATCTGCTTACCTACATTTGGATTCAAGCACATT
                                 I
β6H      AAAATTTCTGCTAATATTGACACACCCGAAGGTGGATTTGATGCAATTATG
          K  I  S  A  N  I  D  T  P  E  G  G  F  D  A  I  M
β6GP     AAAATTTCTGCTAATATTGACAACCCTGAAGGTGGATTCGACGCCATTATG

β6H      CTCCTGGTCTTTGTGAGTGATGCTGATTCTCATTTTGGAATGGACAGCAAA
          L  L  V  F  V  S  D  A  D  S  H  F  G  M  D  S  K
β6GP     CTCCTAGTCTTCGTGAGTGATGCCGATTCTCATTTTGGAATGGACAGCAAA

β6H      AATGAATACTCCATGTCAACTGTCTTGGAATATCCAACAATTGGACAACTC
          N  E  Y  S  M  S  T  V  L  E  Y  P  T  I  G  Q  L
β6GP     AATGAATACTCCATGTCAACTGTCATGGAATATCCAACAATTGGACAACTC
                                    M
β6H      GAACAAGTTCATTTATATGAGAATTACGCAAAACTTATTCCTGGAGCTACA
          E  Q  V  H  L  Y  E  N  Y  A  K  L  I  P  G  A  T
β6GP     GAACAAGTTCCACTATATGAGAATTATGCAAAACTTATTCCTGGAGCCACA
                    P
β6H      GCTTATGAAGAACTGCGGTCTGAGGTGGAACTGGAAGTATTAGGAGACACT
          A  Y  E  E  L  R  S  E  V  E  L  E  V  L  G  D  T
β6GP     GCTTATGAAGAACTGCGGTCTGAGGTGGAGCTGGAAGTATTAGGAGATACA
```

FIG. 3A

```
TCAGTCTTAATGAAAACTTTCTAACTTATATCTCAAGTTTCTTTTCAAAGC  100

ATATTCAGCGTTGGTCTTGTAACGCTGAAGGTAATTCATTTTTTAATCGGT  202

TTTCTATTTCTAGGAAGGAATGATTCACGTACAAGGTGGCTGTGCCTGGGA  304
 F  L  F  L  G  R  N  D  S  R  T  R  W  L  C  L  G      26
TGTGCTCAGGAGAATTTTACTCATCCATCTGGAGTTGGCGAAAGGTGTGAT  406
 C  A  Q  E  N  F  T  H  P  S  G  V  G  E  R  C  D      60
CCTGTCTCCCAAGTAGAAATACTTAAAAATAAGCCTCTCAGTGTAGGCAGA  508
 P  V  S  Q  V  E  I  L  K  N  K  P  L  S  V  G  R      94
AAGTTGAGACCAGGTGGTGCGCAGACTCTGCAGGTGCATGTCCGCCAGACT  610
 K  L  R  P  G  G  A  Q  T  L  Q  V  H  V  R  Q  T     128
GATGACGACCTCAACACAATAAAGGAGCTGGGCTCCGGCCTTTCCAAAGAG  712
 D  D  D  L  N  T  I  K  E  L  G  S  G  L  S  K  E     162
GACGATGACCTCAACACAATCAAAGAGCTGGGCTCCCTGCTTTCAAAGGAG   63
                                   L
AAACCTGTATCCCCTTTTGTGAAAACAACACCAGAAGAAATTGCCAACCCT  814
 K  P  V  S  P  F  V  K  T  T  P  E  E  I  A  N  P     196
AAACCCGTCTCCCCTTTTATGAAAACAACACCAGAGGAAATTGCCAACCCT  165
                   M                                     55
TTGCCATTGACAAATGATGCTGAAAGATTCAATGAAATTGTGAAGAATCAG  916
 L  P  L  T  N  D  A  E  R  F  N  E  I  V  K  N  Q     230
CTGCCATTGACAAATGATGCTGAAAGATTCAATGAAATTGTGAAGAAACAG  267
                                                         89
CAAGCTGCTGTGTGTAAGGAAAAAATTGGCTGGCGGAATGACTCCCTCCAC 1018
 Q  A  A  V  C  K  E  K  I  G  W  R  N  D  S  L  H     264
CAAGCTGCTGTGTGTAAGGAAAAAATTGGCTGGCGGAATGATTCGCTCCAT  369
                                                        123
CTAGCAGGCATCGTCATTCCTAATGACGGGCTCTGTCACTTGGACAGCAAG 1120
 L  A  G  I  V  I  P  N  D  G  L  C  H  L  D  S  K     298
CTGGCAGGCATTGTCATTCCCAACGATGGGCTGTGTCACTTGGACAGCAAG  471
                                                        157
ATTGATAAACTGGTACAAAACAACGTGTTATTGATCTTCGCTGTAACCCAA 1222
 I  D  K  L  V  Q  N  N  V  L  L  I  F  A  V  T  Q     332
ATTGATAAAGTGGTACAAAACAATGTGTTACTGATCTTTGCTGTAACCCAA  573
                                                        191
GTAGGTCTACTTCAGAAGGACTCCGGAAACATTCTCCAGCTGATCATCTCA 1324
 V  G  L  L  Q  K  D  S  G  N  I  L  Q  L  I  I  S     366
GTGGGGCTACTTCACAAGGACTCTGGAAACATTCTCCAACTGATCATCTCA  675
                   H                                    225
GAAGGACTCAACTTGTCATTTACAGCCATCTGTAACAACGGTACCCTCTTC 1426
 E  G  L  N  L  S  F  T  A  I  C  N  N  G  T  L  F     400
GAGGGCCTCAATCTTTCGTTCTCAGCTGTCTGTAACAATGGCACTCTCTTC  777
                                                        259
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FIG. 3B

```
β6H    CAACACCAAAAGAAATGCTCTCACATGAAAGTGGGAGACACAGCTTCCTTC
         Q  H  P  K  K  C  S  H  M  K  V  G  D  T  A  S  F
β6GP   CCACACCAAAAGAAATGCTTGCACATGAAAGTGGGAGAAACAGCTTCATTC
       P                    L                    E
β6H    ATAAAGCCTGTGGGGCTGGGGGATGCCCTGGAATTACTTGTCAGCCCAGAA
         I  K  P  V  G  L  G  D  A  L  E  L  L  V  S  P  E
β6GP   ATAAAGCCTGTGGGGCTGGGGGACACCCTGGAAATCCTTGTCAGCCCAGAA
                                  T                 I
β6H    CACGGGAACGGCTCTTTCCAGTGTGGGGTGTGTGCCTGCCACCCTGGCCAC
         H  G  N  G  S  F  Q  C  G  V  C  A  C  H  P  G  H
β6GP   AATGGGAACGGCTCCTACCAGTGTGGGGTGTGTGCCTGTAACCCAGGCCAC
       N                 Y                    N
β6H    AAGGAGGCCCCAGATCATCCCTCCTGCAGCGGAAGGGGTGACTGCTACTGT
         K  E  A  P  D  H  P  S  C  S  G  R  G  D  C  Y  C
β6GP   AAGGAGACCCCAGACCATCCCTCGTGCAGCGGAAGGGGTGACTGCTACTGT
          T
β6H    TGCCAGTGTGACAATTTCTCCTGCGTGAGACACAAAGGGCTGCTCTGCGGA
         C  Q  C  D  N  F  S  C  V  R  H  K  G  L  L  C  G
β6GP   TGCCAGTGTGACAATTTCTCCTGTGTGAGGCACAAAGGGCTGCTCTGTGGA

β6H    GGCGAGTACTGCAACTGCACCACCAGCACGGACTCCTGCGTCTCTGAAGAT
         G  E  Y  C  N  C  T  T  S  T  D  S  C  V  S  E  D
β6GP   GGAGAGTACTGCAACTGTACCACCAGCACAGACACCTGCATCTCCGAAGAC
                                  T                 I
β6H    ACAAACCCTGGAGCCTCAGGACCAADCTGTGAACGATGTCCTACCTGTGGT
         T  N  P  G  A  S  G  P  T  C  E  R  C  P  T  C  G
β6GP   ACGAACCCTGGAGCCTCGGGACCCACCTGTGAACGATGTCCTACCTGTAGT

β6H    GGCCAAGCCGGAGAAGAATGTGTGGACAAGTGCAAACTAGCTGGTGCGACC
         G  Q  A  G  E  E  C  V  D  K  C  K  L  A  G  A  T
β6GP   GGTCAGCCTGGAGAAGAATGTGTGGACAAATGCAAACTAGCAGGTGTGACC
             P                                       V
β6H    CAAGGAGAAAATGAATGTTTAATTACATTCCTAATAACTACAGATAATGAG
         Q  G  E  N  E  C  L  I  T  F  L  I  T  T  D  N  E
β6GP   CAAGGAGAAAATGAATGTCTTATTACATTCCTAATAAGTACAGATAATGAG
                                                 S
β6H    AACATTCCCATGATCATGTTAGGGGTTTCCCTGGCTACTCTTCTCATCGGG
         N  I  P  M  I  M  L  G  V  S  L  A  T  L  L  I  G
β6GP   AATATTCCTATGATCATGTTGGGGGTTTCACTGGCTA

β6H    GAAGTTGCCAAATTTGAAGCAGAACGATCAAAAGCCAAGTGGCAAACGGGA
         E  V  A  K  F  E  A  E  R  S  K  A  K  W  Q  T  G
β6H    AAACACAGGGAAAAACAAAAGGTAGACCTTTCCACAGATTGCTAGAACTAC
         K  H  R  E  K  Q  K  V  D  L  S  T  D  C
```

FIG. 3C

```
AGCGTGACTGTGAATATCCCACACTGCGAGAGAAGAAGCAGGCACATTATC 1528
 S  V  T  V  N  I  P  H  C  E  R  R  S  R  H  I  I      434
AATGTGACTGTGAGTATACCAAACTGTGAGAGAAAAAGCAGGCATGTTATC  879
 N           S           N           K           V      293
TGCAACTGCGACTGTCAGAAAGAAGTGGAAGTGAACAGCTCCAAATGTCAC 1630
 C  N  C  D  C  Q  K  E  V  E  V  N  S  S  K  C  H      468
TGCAGCTGCGATTGTCAGAAAGAAGTGGAAGTGAACAGCTCCAAATGCCAC  981
    S                                                   327
ATGGGGCCTCGCTGTGAGTGTGGCGAGGACATGCTGAGCACAGATTCCTGC 1732
 M  G  P  R  C  E  C  G  E  D  M  L  S  T  D  S  C      502
ATGGGCCCTCACTGCGAGTGTGGTGAGGACACGCTGAGCACAGATTCCTGC 1083
          H                                             361
GGGCAGTGTATCTGCCACTTGTCTCCCTATGGAAACATTTATGGACCTTAT 1834
 G  Q  C  I  C  H  L  S  P  Y  G  N  I  Y  G  P  Y      536
GGGCAGTGCATCTGCCACTTGTCTCCCTATGGAAACATTTATGGACCTTAC 1185
                                                        395
GGTAACGGCGACTGTGACTGTGGTGAATGTGTGTGCAGGAGCGGCTGGACT 1936
 G  N  G  D  C  D  C  G  E  C  V  C  R  S  G  W  T      570
GATAACGGAGACTGTGAATGTGGGGAATGCGTGTGCAGGAGTGGTTGGACC 1287
 D           E                                          429
GGAGTGCTCTGCAGCGGGCGCGGGGACTGTGTTTGTGGCAAGTGTGTTTGC 2038
 G  V  L  C  S  G  R  G  D  C  V  C  G  K  C  V  C      604
GGCACGCTCTGCAGCGGGCGCGGGGACTGCGTCTGTGGCAAGTGTGTCTGC 1389
    T                                                   463
GACCCCTGTAACTCTAAACGGAGCTGCATTGAGTGCCACCTGTCAGCAGCT 2140
 D  P  C  N  S  K  R  S  C  I  E  C  H  L  S  A  A      638
GACCCCTGTAACTCTAAACGGAGCTGCATTGAATGCCACCTGTCTGCAGAT 1491
                                                 D      497
 S
ATCAGTGAAGAAGAAGATTTCTCAAAGGATGGTTCTGTTTCCTGCTCTCTG 2242
 I  S  E  E  E  D  F  S  K  D  G  S  V  S  C  S  L      672
ATCAGCAAAGAAGCAGATTTCTCAAAGGATAGTTCTGTTTCCTGCTCCCTG 1593
       K     A                 S                        531
GGGAAAACCATCATTCACAGCATCAATGAAAAAGATTGTCCGAAGCCTCCA 2344
 G  K  T  I  I  H  S  I  N  E  K  D  C  P  K  P  P      706
GGAAAAACCATCATTCACAACATCAGTGAAAAAGACTGCCCCAAACCTCCA 1695
                   N     S                              565
GTTGTCCTACTGTGCATCTGGAAGCTACTGGTGTCATTTCATGATCGTAAA 2446
 V  V  L  L  C  I  W  K  L  L  V  S  F  H  D  R  K      740
                                                        1732
                                                        577
ACCAATCCACTCTACAGAGGATCCACAAGTACTTTTAAAAATGTAACTTAT 2548
 T  N  P  L  Y  R  G  S  T  S  T  F  K  N  V  T  Y      774
TTTATGCATAAAAAAAGTCTGTTTCACTGATATGAAATGTTAATG        2644
                                                        788
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FIG. 3D

FIG. 4A

```
β1     human                                MNLQPIFWIGLISSVCCVFAQT
β2     human                                  MLGLRPPLLALVGLLSLGCV
β3     human                              MRARPRPRPLWVTVLALGALAGVGVG
βmyo   Drosophila          MILERNRRCQLALLMIAMLAAIAAQTNAQKAAKLT
β6     human                                MGIELLCLFFLFLGRNDSR

β1       DENRCLKANAKSCGECIQAGPNCGWCTNSTFFQEGMPTSARCDDLEALKKKGCPPDDIENPRGSKDIKKNKNVTNRSKGTAEKLKPEDIHQ 112
β2       LSQECTKFKVSSCRECIESGPGCTWCQKLNFTGPGDPDSIRCDTRPQLLMRGCAADDIMDPTSLAETQEDHNGGQK          QLSPQK 101
β3       GPNICTTRGVSSCQQCLAVSPMCAWCSDEAL    PLGSPRCDLKENLLKDNCAPESIEFPVSEARVLEDRPLSDKGSGDSSQVTQVSPQR 112
βmyo     AVSTCA  SKEKCHTCIQTE GCAWCMQPDF    KGQS RC Y QN TSSLCPEEFAYSPITVEQILVNNKLTNQYKAE insert IVQ 137
β6       TRWLCLG GAETCEDCLLIGPQCAWCAQENFTHPSGVGE RCDTPANLLAKGCQLNFIENPVSQVEILKNKPLSVGRQKNSS     DIVQ 103

β1       IQPQQLVLRLRSGEPQTFTLKFKRAEDYPIDLYYLMDLSYSMKDDLENVKSLGTDLMNEMRRITSDFRIGFGSFVEKTVMPYISTTPAK L 202
β2       VT      LYLRPGQAAAFNVTFRRAKGYPIDLYYLMDLSYSMLDDLRNVKKLGGDLLRALNEITESGRIGFGSFVDKTVLPFVNTHPDK L 186
β3       IA      LRLRPDDSKNFSIQVRQVEDYPVDIYYLMDLSYSMKDDLWSIQNLGTKLATQMRKLTSNLRIGFGAFVDKPVSPYMYISPPEAL 198
βmyo     IQPQSMRLALRVNEKHNIKISYSQAEGYPVDLYYLMDLSKSMEDDKAKLSTLGDKLSETMKRITNNFHLGFGSFVDKVLMPYVSTIPKK L 227
β6       IAPQSLILKLRPGGAQTLQVHVRQTEDYPVDLYYLMDLSASMDDDLNTIKELGSGLSKEMSKLTSNFRLGFGSFVEKPVSPFVKTTPEE I 193
                                          ===B1===

β1       RNPC TSEQNCTTPFSYKNVLSLTNKGEVFNELVGKQRISGNLDSPEGGFDAIMQVAVCGSLIGWRN VTRLLVFSTDAGFHFAGDGKLGG 291
β2       RNPCPNKEKECQPPFAFRHVLKLTNNSNQFQTEVGKQLISGNLDAPEGGLDAMMQVAACPEEIGWRN VTRLLVFATDDGFHFAGDGKLGA 276
β3       ENPCYDMKTTCLPMFGYKHVLTLTDQVTRFNEEVKKQSVSRNRDAPEGGFDAIMQATVCDEKIGWRNDASHLLVFTTDAKTHIALDGRLAG 289
βmyo     EHPC    ENCKAPYGYQNHMPLNNNTESFSNEVKNATVSGNLDAPEGGFDAIMQAIACRSQIGWREQARRLLVFSTDAGFHYAGDGKLGG 314
β6       ANPCSSIPYFCLPTFGFKHILPLTNDAERFNEIVKNQKISANIDTPEGGFDAIMQAAVCKEKIGWRNDSLHLLVFVSDADSHFGMDSKLAG 284
                                                  ===B2====
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FIG. 4B

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β1     IVLPNDGQCHLENNM YTMSHYYDYPSIAHLVQKLSENNIQTIFAVTEEFQPVYKELKNLIPKSAVGTLSANSSNVIQLIIDAYNSLSSEV 381
β2     ILTPNDGRCHLEDNL YKRSNEFDYPSVGQLAHKLAENNIQPIFAVTSRMVKTYEKLTEIIPKSAVGELSEDSSNVVHLIKNAYNKLSSRV 366
β3     IVQPNDGQCHVGSDNHYSASTTMDYPSLGLMTEKLSQKNINLIFAVTENVVNLYQNYSELIPGTTVGVLSMDSSNVLQLIVDAYGKIRSKV 380
βmyo   VIAPNDGECHLSPKGEYTHSTLQDYPSISQINQKVKDNAINIIFAVTASQLSVYEKLVEHIQGSSAAKLDNDSSNVVELVKEEYRKISSSV 405
β6     IVIPNDGLCHLDSKNEYSMSTVLEYPTIGQLIDKLVQNNVLLIFAVTQEQVHLYENYAKLIPGATVGLLQKDSGNILQLIISAYEELRSEV 375

β1     ILENGKLSEGVTISYKSYCKNGVNGTGENGRKCSNISIGDEVQFEISITSNKCPKK D  SDSFKIRPLGFTEEVEVILQYICECECQSEG 469
β2     FLDHNALPDTLKVTYDSFCSNGVTHRNQPRGDCDGVQINVPITFQVKVTATECIQE Q    SFVIRALGFTDIVTVQVLPQCECRCRDQS 452
β3     ELEVRDLPEELSLSFNATCLNNEVIPGL  KSCMGLKIGDTVSFSIEAKVRGCPQE K  EKSFTIKPVGFKDSLIVQVTFDCDCACQAQA 466
βmyo   EMKDNATGD VKITYFSSCLSNGPEVQT  SKCDNLKEGQQVSFTAQIQLLKCPEDPRDWTQTIHISPVGINEVMQIQLTMLCSCPCENPG 493
β6     ELEVLGDTEGLNLSFTAICNNGTLFQHP  KKCSHMKVGDTASFSVTVNIPHC ER R  SRHIIIKPVGLGDALELLVSPECNCDCQKEV 460

β1     I    PESPKCHEGNGTFECGACRCNEGRVGRHCECSTDEVN SEDM DAYCRKENSS EICSNNGECVCGQCVCRKRDNTNEIYSGKFCE 553
β2     R     DRSLCH GKGFLECGICRCDTGYIGKNCECQTQGRS SQEL EGSCRKDNNS IICSGLGDCVCGQCLCHTSDVPGKLIYGQYCE 534
β3     E    PNSHRCNNGNGTFECGVCRCGPGWLGSQCECSEEDYRPSQQ  DE CSPREGQ PVCSQRGECLCGQCVCHSSDF GKIT GKYCE 547
βmyo   SIGYQVQANSCS GHGTSMCGICNCDDSYFGNKCECSATDLT SKFANDTSCRADSTSTTDCSGRGHCVCGACECHKRPNPIEIISGKHCE 582
β6     E    VNSSKCHHGNGSFQCGVCACHPGHMGPRCECGEDML  ST   D SCKEAPDH PSCSGRGDCYCGQCICHLSPY GN IYGPYCQ 538
                                                                          ===B3===
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β1   CDNFNCDRSNGLICGG   NGVCKCRVCECNPNYTGSACDCSLDTSTCEASN GQICNGRGICECGVCKCT   DPKFQGQTCEMCQTCLGV 638
β2   CDTINCERYNGQVCGGPGRGLCFCGKCRCHPGFEGSACQCERTTEGCLNPR RVECSGRGRCRCNVCECH   SG YQLPLCQECPGCPSP 620
β3   CDDFSCVRYKGEMCSG   HGQCSCGDCLCDSDWTGYYCNCTTRTDTCMSSN GLLCSGRGKCECGSCVCI   QPGSYGDTCEKCPTCPDA 632
βmyo CDNFSCERNRNQLCSGPDHGTCECGRCKCKPGWTGSNCGCQESNDTCMPPGGGEICSGHGTCECGVCKCTVNDQGRFSGRHCEKCPTCSGR 673
β6   CDNFSCVRHKGLLCGG   NGDCDCGECVCRSGWTGEYCNCTTSTDSCVSED GVLCSGRGDCVCGKCVCT   NPGASGPTCERCPTCGDP 623

β1   CAEHKECVQCRAFNKGE  KKDTCTQECSYFNITKVESRDKLPQPVQPDPVSHCKEKDVDDCWFYFTY SVNGNNEVMVHVVENPECPTGP 726
β2   CGKYISCAECLKFEKGPF  GKNCSAACPG     LQLSN      NPVKGRT CKERDSEGCWVAYTLEQQDGMDRYLIYVDESRECVAGP 698
β3   CTFKKECVECKKFDREPYMTENTCNRYCRDEIESVKELKD     TGKDAVN CTYKNEDDCVVRFQY YEDSSGKSILYVVEEPECPKGP 715
βmyo CQELKDCVQCQMYKTGELKNGDDCARNCTQFVPVGVEKVEID   ETKDEQM CKFFDEDDCKFMFKY   SEQGELHVYAQENKECPAKV 757
β6   CNSKRSCIECHLSAAGQA  GEECVDKCKLAGATISEEEDF    SKDGSVS CSLQGENECLITFLI TTDNEGKTIIHSINEKDCPKPP 706

β1   DIIPIVAGVVAGIVLIGLALLLIWKLLMIIHDRREFAKFEKEKMNAKWDTGENPIYKSAVTTVVNPKYEGK             797
β2   NIAAIVGGTVAGIVLIGILLLVIWKALIHLSDLREYRRFEKEKLKSQWNN DNPLFKSATTTVMNPKFAES             769
β3   DILVVLLSVMGAILLIGLAALLIWKLLITIHDRKEFAKFEEERARAKWDTANNPLYKEATSTFTNITYRGT             786
βmyo FMLGIVMGVIAAIVLVGLAILLLWKLLTTIHDRREFARFEKERMNAKWDTGENPIYKQATSTFKNPMYAGK             828
β6   NIPMIMLGVSLATLLIGVVLLCIWKLLVSFHDRKEVAKFEAERSKAKWQTGTNPLYRGSTSTFKNVTYKHREKQKVDLSTDC 788
                ====B4====
```

FIG. 4C

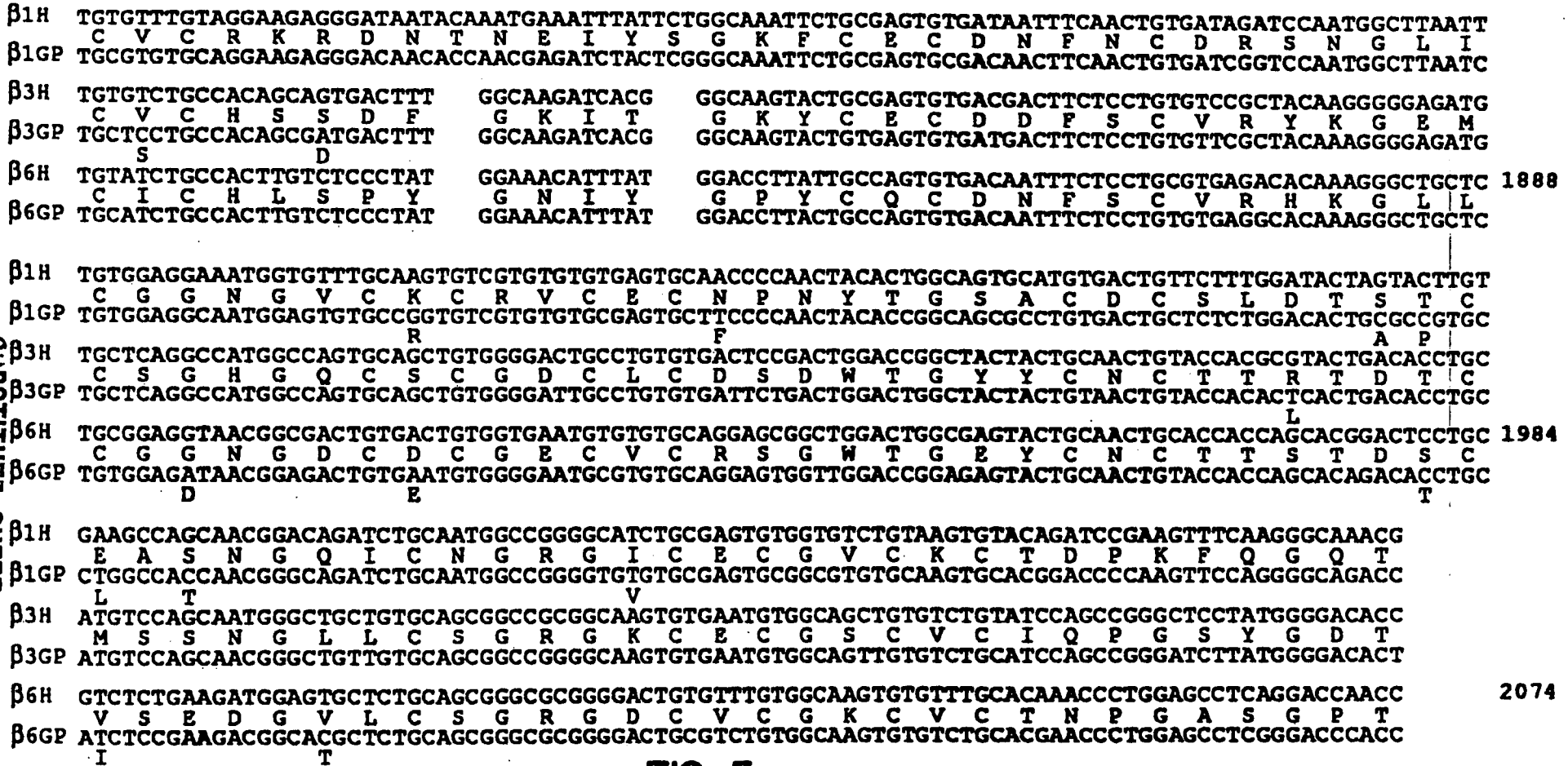

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/00236

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12N 5/10,15/09,15/11,15/12,15/03,15/07; G01N 33/566;C07K 15/06,15/14 15/24,15/28 US Cl: 530/350,395,387; 536/27; 435/320.1,252.3,240.2,69.1;436/503

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S. Cl.	530/350,395,387; 536/27; 435/320.1, 252.3,240.2; 69.1; 436/503

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

APS and DIALOG Files 155,5,399, WPI, 35, 340 and 357 for integrin and receptor and (B6 or beta 6) and N-terminal sequences.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	The Journal of Biological Chemistry. Vol. 265. No. 20. issued 15 July 1990. Sheppard et al. "Complete Amino Acid Sequence of a Novel integrin B Subunit (B6) identified in Epithelial Cells Using the Polymerase Chain Reaction. pages 11502-11507. See whole publication: especially the abstract and p. 11505 and 11506.	1-13 1-21
X Y	The EMBO Journal. Vol. 8. No 10. issued 1989. Freed et al.. "A Novel integrin Beta subunit is Associated with the Vitronectin Receptor Alpha Subunit (alpha) is a Human Osteosarcoma Cell Line and is a Substrate for Protein Kinase C". pages 2955-2965. See whole publication; especially the abstract.	1-5 1-21

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
23 APRIL 1991	22 MAY 1991
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	NGUYEN T. C-HO INTERNATIONAL DIVISION Keith C. Furman for

Form PCT/ISA/210 (second sheet) (May 1986)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Cell . Vol. 44. Issued 28 February 1986. Ruoslahti et al. Arg-Gly-Asp: A Versatile Cell Recognition Signal". pages 517 and 518.	14-21